

PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL EUSTRONGYLIDES. XI. INFLUENCE OF OXYGEN TENSION ON THE AEROBIC AND POST-ANAEROBIC OXYGEN CONSUMPTION.

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Harnisch (1932a, b; 1933; 1935a, b, c) has emphasized that the respiration of parasitic worms is dependent on the oxygen tension at least up to a tension of 760 mm. Hg. He has also shown that the post-anaerobic oxygen consumption, the repayment of an oxygen debt, shows the same relationship in a number of free living organisms, the normal aerobic respiration of which is much more independent on the tension. He has used this argument as one of the cornerstones for his hypothesis that the respiration of parasitic worms and the post-anaerobic oxygen consumption of free living animals is governed by another set of enzymes (secondary aerobiosis) than the normal aerobic respiration (primary aerobiosis) of the latter. Von Buddenbrock (1939) has questioned this view in so far as free living animals are concerned, pointing out that the tension at which the oxygen consumption begins to decline is not static but changes with the intensity of the oxygen consumption.

Since Harnisch used for his investigations on parasites only intestinal and bile-duct helminths, it appeared worthwhile to study in this respect the larvae of *Eustrongylides ignotus* which definitely lead in nature a much more aerobic life than the former (von Brand, 1942). It was hoped to decide between Harnisch' and von Buddenbrock's views by using larvae at various levels of metabolism, the changes being partly induced by the stimulating effect of the potassium ion (von Brand, 1943), partly by various temperatures. Although this hope was not completely fulfilled, the experiments in themselves are of sufficient interest to warrant publication.

MATERIAL AND METHODS

In the respiration experiments reported upon previously in this series (von Brand, 1942, 1943) *Eustrongylides* larvae freshly isolated from the cysts which they normally occupy in *Fundulus* were used almost exclusively. It was then found that they showed initially a trace of excess oxygen consumption. This phase had to be eliminated for the present experiments. The worms were therefore isolated in batches from 3 to 5 specimens, weighed, and kept over night in a shallow layer of either 0.85 per cent NaCl or 1.14 per cent KCl, that is, under conditions allowing for a completely aerobic metabolism. After this preliminary period their oxygen consumption was studied at various oxygen tensions with a minimum of 1½ hour for each tension. The fluid in the respiration vessels was the same as that used during

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the preliminary period. The oxygen determinations were carried out by means of a Warburg apparatus. The gas mixtures were passed for 20 minutes through the vessels before closing the manometers; they contained oxygen at the following tensions: 760, 160, 34, and 6 mm. Hg. Usually from 2 to 3 different tensions were used consecutively with one batch of worms. Care was taken to employ the highest tension first in order to avoid an excessively high oxygen consumption if the worms should acquire an oxygen debt at the lower tensions. Previous experiments had shown that the respiration of the larvae remains virtually constant over long periods if the conditions are not changed. Any decline in oxygen consumption following the replacement of a gas mixture with high oxygen tension by one containing less oxygen could consequently safely be ascribed to the lowered tension. The experiments of these series were carried out at 40.2, 28.5, and 18.8° C.

A slightly different procedure had to be employed in the series in which the dependency of the post-anaerobic oxygen consumption on the tension was studied. The isolated and weighed worms were first exposed, in 0.85 per cent NaCl, for a period of 16 hours to anaerobic conditions, using the method previously described (von Brand, 1942). In order to get as uniform an oxygen debt as possible, the worms were subjected to this anoxic period at 37° C. regardless of the temperature at which the post-anaerobic oxygen consumption was studied subsequently. The repayment of an oxygen debt is a transitory phenomenon; after an initial high rate, the oxygen consumption declines slowly until it reaches finally the pre-anaerobic level. The phase of high oxygen consumption will last longer at low than at high temperatures if an equal oxygen debt is repaid at both temperatures. These facts precluded obviously the consecutive use of various gas mixtures with one and the same group of animals. Instead, a new batch of worms was used for each experiment at each tension and only the oxygen consumed during the first half hour was taken into consideration. These experiments were carried out at the same tensions and temperatures as those mentioned above; an additional series was performed at 9.8° C.

## RESULTS AND DISCUSSION

A summary of the experiments is presented in Table I. It is evident that the dependency of the oxygen consumption on the tension is much more pronounced at high than at low temperatures if the comparison is made between series performed under otherwise equal conditions. In the aerobic NaCl series, for example, the oxygen consumption was dependent, at 40.2° C., on the tension over the entire range of tensions studied, while at 18.8° C., it was almost constant in the range of 760 to 34 mm. Hg.

The post-anaerobic oxygen consumption was, at each temperature studied, about three times as high as the normal aerobic respiration in NaCl solution when the values found at an oxygen tension of 760 mm. Hg are compared.

If, as von Buddenbrock (1939) maintains, the intensity of the respiratory rate decides at what tension the consumption begins to decline, one should expect that at each temperature the curve for the post-anaerobic oxygen consumption should be steeper than that of either the aerobic NaCl or KCl series. The observations confirm, on the whole, this view, especially at tensions below 160 mm. Hg. Between 760 and 160 mm. Hg there was hardly a significant difference between the three

TABLE I  
*Influence of oxygen tension on the normal aerobic and the post-anaerobic oxygen consumption of larval Eustrongylides ignotus.*  
*Under oxygen consumption the mean values with the standard error of the means and, in brackets, the extremes are listed*

Oxygen tension, mm. Hg	Temp. °C.	Aerobic, 0.85 per cent NaCl				Aerobic, 1.14 per cent KCl				Post-anaerobic, 0.85 per cent NaCl			
		760	160	34	6	760	160	34	6	760	160	34	6
Experiments, No.	40.2	14	19	12	8	7	15	8	12	8	8	8	8
O <sub>2</sub> consumption mm. <sup>3</sup> /g./half hour	139±11 (75, 216)	104±5 (69, 140)	87±4 (62, 110)	27±2.2 (19, 36)	174±9 (137, 214)	148±9 (100, 214)	100±9 (62, 149)	22±2.8 (11, 37)	373±7 (349, 412)	318±25 (246, 468)	131±15 (68, 204)	38±4.8 (16, 58)	30±4.4 (9, 47)
Experiments, No.	28.5	16	24	14	20	15	16	15	15	12	12	10	10
O <sub>2</sub> consumption mm. <sup>3</sup> /g./half hour	67±3.3 (43, 88)	51±3.2 (31, 84)	48±3.9 (30, 73)	32±2.5 (10, 52)	86±3.7 (63, 107)	69±4.5 (39, 117)	70±3.0 (53, 89)	27±3.0 (10, 51)	196±5 (164, 224)	177±6 (161, 215)	91±7.7 (42, 132)	30±4.4 (9, 47)	30±4.4 (9, 47)
Experiments, No.	18.8	12	12	12	12	12	12	12	12	12	12	11	11
O <sub>2</sub> consumption mm. <sup>3</sup> /g./half hour	30±1.9 (16, 39)	29±2.3 (19, 42)	28±1.7 (19, 40)	13±0.8 (8, 20)	41±2.5 (25, 54)	38±1.7 (29, 48)	37±2.9 (22, 54)	13±2.1 (5, 28)	87±2.9 (76, 101)	83±3.6 (71, 97)	61±2.3 (48, 72)	12±2.0 (5, 23)	12±2.0 (5, 23)
Experiments, No.	9.8									10	12	12	12
O <sub>2</sub> consumption mm. <sup>3</sup> /g./half hour										46±1.9 (38, 57)	43±1.9 (33, 52)	39±1.5 (27, 48)	11±1.2 (4, 18)

conditions studied, with the exception of the 40.2° series which showed the postulated relationship. To make von Buddenbrock's view of the essential similarity between normal and post-anaerobic oxygen consumption entirely acceptable, another prerequisite would have to be met. One should expect that the dependency of the post-anaerobic oxygen consumption on the tension should be the same as the aerobic one if one compares series in which the intensity was equal at a tension of 760 mm. Hg, i.e., if one takes as basis of comparison post-anaerobic experiments performed at lower temperatures than the aerobic ones. The post-anaerobic experiments performed at 18.8° C. showed at 760 mm. Hg exactly the same intensity in oxygen consumption as the 28.5° C. KCl series. Similarly, the rate of oxygen consumption in the 9.8° C. post-anaerobic series was quite similar to that found in the 18.8° C. KCl series. In both cases as slightly greater dependency on the tension was observed in the post-anaerobic series; the differences are, however, not statistically significant.

While the present experiments seem, therefore, to support von Buddenbrock's view, they do not necessarily as yet completely disprove Harnisch's conception of "primary" and "secondary aerobiosis." Harnisch has found evidence that the respiration of parasitic worms is almost exclusively of the second type, that is, it corresponds to the post-anaerobic oxygen consumption of free living animals. One would then also expect identical curves in cases where the metabolic levels coincide. Although the larvae of *Eustrongylides ignotus* resemble in their general metabolism unquestionably free living animals more than they do intestinal helminths, it cannot be stated categorically that they do so in the question under consideration. To decide definitely between Harnisch and von Buddenbrock, experiments similar to those described in the present paper should be performed on free living animals. Certainly, von Buddenbrock's criticism of Harnisch's failure to consider the intensity factor is justified, but that is hardly sufficient to reject the latter's views without further experimentation.

One further point deserves brief discussion. Von Buddenbrock (1939) has pointed out that the reasons for assuming a dependency of the oxygen consumption on the tension are occasionally rather insecure because the gases going into purely physical solution are neglected. He cites the example of the sea urchin *Sphaerechinus granularis* which shows for a certain period an increased oxygen consumption if it is transferred from sea water with normal oxygen content into water containing an abnormally high oxygen content. Analyses of the coelomic fluid revealed that the greatest part of this excess oxygen had simply gone into physical solution and had not participated in the metabolic processes. Such an explanation cannot be applied to the *Eustrongylides* larvae. Some of the worms were, after their metabolic level had been ascertained at an oxygen tension of 160 mm. Hg, kept up to 6 hours in pure oxygen at 40.2° C. They showed during the entire time a regular oxygen consumption which was all the time higher than that found in the preliminary period. Obviously, we are dealing here with a case of true dependency on the tension, just as it occurs in actinians which are, as is known since Henze's (1909) investigation, a classical example for this type of relationship.

It should finally be noted that the exposure to pure oxygen for 6 hours did not seem to harm the *Eustrongylides* larvae. They differ in this respect from the intestinal helminth *Ascaris* which is rapidly killed by oxygen of high tension (Laser,

1944). This difference may have a biological basis in the fact that the latter worm lives normally in a much oxygen poorer habitat than the former.

#### SUMMARY

1. The oxygen consumption of larval *Eustrongylides ignotus* shows a greater dependency on the oxygen tension at high than at low temperature.

2. The post-anaerobic oxygen consumption is more dependent on the tension than the respiration of larvae that were exposed previously to well oxygenated surroundings, if experiments performed at equal temperatures are compared. This difference disappears almost completely, however, if experiments are compared in which the intensity of the oxygen consumption was equal in both sets at a tension of 760 mm. Hg.

3. The implication of these data is discussed on the controversy between Harnisch and von Buddenbrock as to whether it is justified to distinguish between "primary" and "secondary aerobiosis."

4. The dependency of the oxygen consumption on the tension is a true one and is not only simulated by oxygen going into physical solution in the body fluids at higher tensions.

5. The larvae of *Eustrongylides*, in contrast to *Ascaris*, are not killed by a 6-hour exposure to pure oxygen.

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